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### (54) Enzyme product and method for improving bread quality

Enzymprodukt und Verfahren zur Verbesserung der Qualität von Brot

Produit enzymatique et méthode d'amélioration de la qualité du pain

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## Description

The present invention relates to a bread improver composition and to a method of using the composition to improve bread quality.

5 Bread improvers are complex mixtures containing various functional ingredients such as oxidizing and reducing agents (e.g. ascorbic acid, cysteine), enzymes (e.g.  $\alpha$ -amylase, hemicellulase), emulsifiers (e.g. DATA-ester, monoglycerides, SSL), fatty materials (e.g. fat, lecithin) and carriers or bulk materials (starch, sugars, etc). Many of the commonly used bread improvers contain emulsifiers which are used for improving dough strength and crumb softness. From the consumer's point of view it is advantageous to minimize the use of emulsifiers, which are considered as chemical addi-  
 10 tives. The resistance of consumers to chemical additives is growing and there is therefore constant need to replace emulsifiers by consumer friendly additives and/or enzymes, which are considered as processing aids. However, bread quality is lowered considerably when emulsifiers are omitted, for example, it is difficult to achieve a shelf life of 3 to 5 days for non-crusty types of bread such as sandwich breads without using emulsifiers like SSL or monoglycerides.

Studies on bread staling have indicated that the starch fraction in bread recrystallizes during storage, thus causing  
 15 an increase in crumb firmness. Amylases and hemicellulases are widely used in bread improvers to improve crumb softness and loaf volume.  $\alpha$ -Amylases partially degrade the starch fraction during baking and increase crumb softness. Hemicellulases break down the hemicellulose fraction of wheat flour, thus releasing water normally bound to this fraction into the dough. The use of hemicellulases in bread improvers results in an improved oven spring of the dough during baking, an improved loaf volume, grain structure and better keeping quality of the baked bakery product. However, the  
 20 combined improvements imparted by amylases and hemicellulases are limited and therefore emulsifiers are still required for obtaining an acceptable keeping quality of bread.

Lipases are rarely used in bread improvers and detrimental effects have been observed from the action of endogenous lipase liberating unsaturated fatty acids into the dough (Pyler 1988).

Surprisingly it has been found that the combined use of a hemicellulase, an amylase, preferably an  $\alpha$ -amylase, a  
 25 lipase and preferably shortening has a complementary synergistic effect in such a way that loaf volume and crumb softness of the bread is clearly better than when each of the enzymes is used individually. The excellent crumb softness of bread obtained by addition of this combination of enzymes and shortening allows a complete elimination of emulsifiers without lowering the quality of the bread.

The present invention provides a bread improver composition which comprises at least one lipase, at least one  
 30 hemicellulase and at least one amylase.

The present invention further provides a dough which comprises the bread improver composition, flour, water and yeast.

The dough of the invention may be baked to produce bread of improved quality and crumb softness.

The dough may further comprise shortening, preferably 100 g or less of shortening per kilogram flour, for example  
 35 15 to 50 g of solid shortening per kilogram flour.

The dough may further comprise oxidizing and reducing agents. Preferably ascorbic acid is used as an oxidant in amounts of 0.15 g or less per kg of flour. Preferably L-cysteine is used as a reducing agent in amounts less than 0.05 g per kg of flour.

The dough of the invention may also comprise salt, sugar, or other normal dough ingredients.

40 The dough of the present invention is prepared without adding conventional emulsifiers such as monoglycerides (GMS), diacetyl tartaric acid esters of mono- and diglycerides (DATA-esters) and the sodium or calcium salt of stearic acids (SSL/CSL).

The amounts of the various enzymes to be included in the dough vary depending on various factors such as enzyme activity, baking method, kind of bread, fermentation time and temperature and the kind of raw materials used. It will be  
 45 appreciated that the skilled person is able without undue experimentation to determine the effective amounts of the enzymes in question.

According to the present invention the dough comprises from 25-1250 FAU fungal amylase units (FAU) per kg flour, more preferably between 75-250 FAU per kg flour. The amylase is generally fungal amylase for example from a strain of *Aspergillus oryzae*. The fungal amylase activity is measured at pH = 5.5 and 30°C using Phadebas tablets (Pharmacia)  
 50 as a substrate and a fungal amylase preparation of 10 000 FAU/g as an internal reference [1 F(ungal) A(mylase) U(nit) is equivalent to 10 SKB-units].

According to the present invention hemicellulase is added preferably in an amount varying between 25-500  $\beta$ -xylanase units per kg flour, more preferably between 35-280  $\beta$ -xylanase units per kg flour. The hemicellulase is preferably fungal hemicellulase, for example from *Aspergillus* or *Trichoderma* strains. The hemicellulase activity is determined at  
 55 pH = 4.7 and 40°C on a dyed xylan substrate (Xylazyme tablets from MegaZyme Inc. Australia). One  $\beta$ -xylanase unit is defined as the amount of enzyme required to release one micromole of xylose reducing equivalents per minute under the defined assay conditions.

According to the present invention lipase is added preferably in an amount varying between 400-4000 lipase units per kg of flour, more preferably between 800-2000 lipase units per kg of flour.

The lipase is preferably fungal lipase produced by a Rhizopus, Aspergillus, Candida, Penicillium or Mucor strain. Preferably a lipase from a strain of Rhizopus arrhizus or Rhizopus oryzae is used. The lipase activity is determined in a titrimetric test method at 37°C and pH = 6.0 using an oil in water emulsion of olive oil as a substrate. One lipase unit is defined as the amount of enzyme required to liberate one micromole of fatty acid per minute under the defined assay conditions.

The present invention will be further demonstrated by the following examples. It should be noted that the present invention is by no means limited to these examples.

#### EXAMPLE 1

##### Preparation of white pan bread (no-time straight dough method) and measurement of crumb firmness

For bread making a dough was prepared from 3500 g of flour (100%), 1960 ml water (55%), 87.5 g compressed yeast (2.5%), 52.5 g sugar (1.5%), 70 g salt (2%), 0-175 g of shortening (0-5%), 105 mg ascorbic acid (30 ppm), 70 mg L-cysteine (20 ppm) and various quantities of the enzymes indicated in the Tables. The ingredients were mixed into a dough using a Kemper spiral mixer (350 rotations at speed 1 followed by 1200 rotations at speed 2).

Dough pieces of 900 g were rounded by hand, proofed for 35 minutes at 30°C, punched, moulded, panned, proofed for 65 minutes at 34°C and baked for 30 minutes in an oven at 220°C. Loaf volume was determined by rapeseed displacement method. After storing the loaves for three days in polyethylene bags at room temperature crumb firmness was measured using a Stevens Texture Analyser. Two slices of 2 cm thickness from the center of each loaf were analysed by the texture analyser using a probe of 1.5 inch diameter, a compression depth of 5 mm (25%) and a rate of compression of 0.5 mm/sec.

Table 1 shows the results of baking test using various amounts of shortening, emulsifier (distilled monoglycerides), hemicellulase, lipase and amylase. It appears from Table 1 that addition of a combination of shortening,  $\alpha$ -amylase, hemicellulase and lipase results in excellent bread quality and superior crumb softness. The crumb softness obtained with this combination is superior to the crumb softness obtained with 0.5% distilled monoglycerides. It is apparent,

therefore, that excellent bread quality is obtained when using this enzyme combination without an emulsifier.

Table 1

Addition	loaf volume (ml)	crumb firmness after 72 hours (g)
1. shortening (0%)	3500	900
2. shortening (0%) distilled mono- glycerides (0.5%)	3500	720
3. shortening (0%) lipase (2000 U/kg flour)	3200	950
4. shortening (0%) fungal amylase (90 FAU/kg flour) hemicellulase (105 U/kg flour)	3800	650
5. shortening (0%) fungal amylase (90 FAU/kg flour) hemicellulase (105 U/kg flour) lipase (2000 U/kg flour)	3300	950
6. shortening (3%)	3600	710
7. shortening (3%) distilled mono- glycerides (0.5%)	3600	550
8. shortening (3%) lipase (2000 U/kg flour)	3600	560
9. shortening (3%) fungal amylase (90 FAU/kg flour) hemicellulase (105 U/kg flour)	4050	450
10. shortening (3%) fungal amylase (90 FAU/kg flour) hemicellulase (105 U/kg flour) lipase (2000 U/kg flour)	4100	350

## EXAMPLE 2

Baking tests were carried out as described in Example 1. A recipe containing 3% shortening was used throughout this baking test and crumb firmness of the breads was measured as described in Example 1.

Table 2 shows the result of the baking test using various emulsifiers and enzyme combinations. Superior bread quality is obtained when adding a combination of amylase, hemicellulase and lipase. This Example demonstrates that bread improvers containing this enzyme combination have better performance than bread improvers containing conventional emulsifiers like SSL, DATA-esters or monoglycerides.

Table 2

Addition	loaf volume (ml)	crumb firmness after 72 hours (g)
1. fungal amylase (30 FAU/kg flour) hemicellulase (35 U/kg flour) distilled mono- glycerides (0.1%)	3700	580
2. fungal amylase (90 FAU/kg flour) hemicellulase (105 U/kg flour) distilled mono- glycerides (0.45%)	3900	400
3. fungal amylase (30 FAU/kg flour) hemicellulase (35 U/kg flour) SSL (0.1%)	3850	520
4. fungal amylase (90 FAU/kg flour) hemicellulase (105 U/kg flour) SSL (0.3%)	3950	415
5. fungal amylase (30 FAU/kg flour) hemicellulase (35 U/kg flour) DATA-ester (0.1%)	3950	500
6. fungal amylase (90 FAU/kg flour) hemicellulase (105 U/kg flour) DATA-ester (0.3%)	4000	410
7. fungal amylase (30 FAU/kg flour) hemicellulase (35 U/kg flour) lipase (1000 U/kg flour)	3900	450
8. fungal amylase (90 FAU/kg flour) hemicellulase (105 U/kg flour) lipase (3000 U/kg flour)	4100	320

## EXAMPLE 3

Breads were baked with variable amounts of shortening and enzyme combination comprising fungal amylase (90 FAU/kg flour), hemicellulase (105 U/kg flour) and lipase (3000 U/kg flour). The results (see Table 3) show that the amount of shortening in the recipe is critical for obtaining the desired bread quality. Best results are obtained when using high levels of shortening, whereas solid shortening gives better results than liquid oil.

Table 3

Addition	loaf volume (ml)	crumb firmness after 72 hours (g)
1. shortening (0%)	3250	900
2. shortening (1%)	3900	450
3. soy bean oil (1%)	3400	790
4. shortening (3%)	4100	350
5. shortening (5%)	4050	330

## EXAMPLE 4

White pan bread was prepared according to the following recipe for a sponge and dough process:

Sponge	Flour	70 %
	Yeast	2 %
	Yeast food	0.3%
	Water	38.5%
Dough	Flour	30 %
	Water	16.5%
	Sugar	5 %
	Salt	2 %
	Milkpowder	4 %
	Shortening	5 %

The sponge was prepared by mixing the ingredients into a dough using a Kemper spiral mixer (380 rotations at speed 1 followed by 700 rotations at speed 2). After a sponge fermentation of 4 hours at 28°C the sponge was returned into the mixer bowl, all of the dough ingredients were added and mixed into a dough (280 rotations at speed 1 followed by 700 rotations at speed 2 in the Kemper spiral mixer).

The dough of 29°C temperature was divided immediately after mixing into pieces of 620 g which were proofed during 25 minutes at 31°C, moulded, panned, proofed for 65 minutes at 43°C and baked for 25 minutes at 190°C. Crumb firmness was measured after 3 days storage according to the procedure described in Example 1. The results (Table 4) show that superior bread quality is obtained in a sponge and dough process, when adding a combination of lipase,

hemicellulase and amylase at this dough site.

Table 4

Addition (at dough site)	loaf volume (ml)	crumb firmness after 72 hours (g)
1. fungal amylase (112.5 FAU/kg flour)	2875	530
2. fungal amylase (112.5 FAU/kg flour) distilled monoglycerides (0.3%)	2870	440
3. fungal amylase (112.5 FAU/kg flour) hemicellulase (112 U/kg flour)	2950	480
4. fungal amylase (112.5 FAU/kg flour) hemicellulase (112 U/kg flour) lipase (1200 U/kg flour)	3000	360

### Claims

1. A bread improver composition which comprises at least one lipase, at least one hemicellulase and at least one amylase.
2. A composition according to claim 1 wherein the lipase is derived from a Rhizopus, Aspergillus, Candida, Penicillium or Mucor strain.
3. A composition according to claim 1 or 2 wherein the hemicellulase is derived from an Aspergillus or Trichoderma strain.
4. A composition according to any one of the preceding claims wherein the amylase is an  $\alpha$ -amylase and preferably derived from Aspergillus oryzae.
5. A dough which comprises a composition as claimed in any one of the preceding claims, flour, water and yeast.
6. A dough according to claim 5 which further comprises shortening, preferably comprises 100 g or less of shortening per kg of flour and more preferably comprises from 15 to 30 g shortening per kg of flour.
7. A dough according to any one of claims 5 or 6 which comprises from 25 to 1250 fungal amylase units (FAU) of  $\alpha$ -amylase per kilogram of flour and preferably comprises from 75-250 FAU of  $\alpha$ -amylase per kilogram of flour.
8. A dough according to any one of claims 5 to 7 which comprises from 25 to 500  $\beta$ -xylanase units of hemicellulase per kilogram of flour and preferably comprises from 35 to 250  $\beta$ -xylanase units of hemicellulase per kilogram of flour.
9. A dough according to any one of the preceding claims which comprises from 450 to 4000 units lipase per kilogram of flour and preferably comprises from 500 to 2500 units lipase per kilogram of flour.
10. A dough according to any one of the preceding claims which is substantially free of emulsifiers.
11. A process for producing bread which comprises forming a dough as claimed in any one of claims 5 to 10 and baking the dough.

### Patentansprüche

1. Backhilfsmittel, welches mindestens eine Lipase, mindestens eine Hemicellulase und mindestens eine Amylase umfasst.
2. Mittel nach Anspruch 1, in welchem die Lipase von einem Rhizopus-, Aspergillus-, Candida-, Penicillium- oder Mucor-Stamm abgeleitet ist.

3. Mittel nach Anspruch 1 oder 2, in welchem die Hemicellulase von einem Aspergillus- oder Trichoderma-Stamm abgeleitet ist.
- 5 4. Mittel nach einem der vorhergehenden Ansprüche, in welchem die Amylase eine  $\alpha$ -Amylase und vorzugsweise von Aspergillus oryzae abgeleitet ist.
5. Teig, welcher Mehl, Wasser, Hefe und ein Mittel nach einem der vorhergehenden Ansprüche umfasst.
6. Teig nach Anspruch 5, welcher ausserdem einen Fettstoff, vorzugsweise 100 g Fett oder weniger per kg Mehl und  
10 noch bevorzugter 15 bis 30 g Fett per kg Mehl enthält.
7. Teig nach einem der Ansprüche 5 und 6, welcher 25 bis 1250 Pilzamyase-Einheiten (PAE) der  $\alpha$ -Amylase per kg Mehl und vorzugsweise 75 bis 250 PAE der  $\alpha$ -Amylase per kg Mehl enthält.
- 15 8. Teig nach einem der Ansprüche 5 bis 7, welcher 25 bis 500  $\beta$ -Xylanase-Einheiten der Hemicellulase per kg Mehl und vorzugsweise 35 bis 250  $\beta$ -Xylanase-Einheiten der Hemicellulase per kg Mehl enthält.
9. Teig nach einem der vorhergehenden Ansprüche, welcher 450 bis 4000 Lipase-Einheiten per kg Mehl und vorzugsweise 500 bis 2500 Lipase-Einheiten per kg Mehl enthält.
- 20 10. Teig nach einem der vorhergehenden Ansprüche, welcher im wesentlichen von Emulgatoren frei ist.
11. Verfahren zur Brotherstellung, welches das Herstellen eines Teigs nach einem der Ansprüche 5 bis 10 und das Backen des Teigs umfasst.

25  
Revendications

1. Composition d'amélioration du pain, qui comprend au moins une lipase, au moins une hémicellulase et au moins une amylase.
- 30 2. Composition suivant la revendication 1, dans laquelle la lipase provient d'une souche de Rhizopus, Aspergillus, Candida, Penicillium ou Mucor.
3. Composition suivant la revendication 1 ou 2, dans laquelle l'hémicellulase provient d'une souche d'Aspergillus ou de Trichoderma.
- 35 4. Composition suivant l'une quelconque des revendications précédentes, dans laquelle l'amylase est une  $\alpha$ -amylase et provient, de préférence, de Aspergillus oryzae.
- 40 5. Pâte qui comprend une composition suivant l'une quelconque des revendications précédentes, de la farine, de l'eau et de la levure.
6. Pâte suivant la revendication 5, qui comprend, en outre, une matière grasse, de préférence, 100 g ou moins de matière grasse par kg de farine et, avec avantage, de 15 à 30 g de matière grasse par kg de farine.
- 45 7. Pâte suivant l'une quelconque des revendications 5 ou 6, qui comprend de 25 à 1250 unités amylase fongique (UAF) d' $\alpha$ -amylase par kg de farine et de préférence, qui comprend de 75 à 250 UAF d' $\alpha$ -amylase par kg de farine.
8. Pâte suivant l'une quelconque des revendications 5 à 7, qui comprend de 25 à 500 unités  $\beta$ -xylanase d'hémicellulase par kg de farine et de préférence, qui comprend de 35 à 250 unités  $\beta$ -xylanase d'hémicellulase par kg de farine.
- 50 9. Pâte suivant l'une quelconque des revendications précédentes, qui comprend de 450 à 4000 unités lipase par kg de farine et de préférence, qui comprend de 500 à 2500 unités lipase par kg de farine.
10. Pâte suivant l'une quelconque des revendications précédentes, qui est sensiblement exempte d'agents émulsifiants.
- 55 11. Procédé de préparation de pain, qui comprend la formation d'une pâte suivant l'une quelconque des revendications 5 à 10 et la cuisson de la pâte.